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Re-collection of secotioid *Entoloma calongei* in Europe

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Abstract—In 2017, the secotioid *Entoloma calongei* was recorded in Slovakia, the first report since its Spanish holotype collection. Morphological and ecological characteristics are presented and compared with the type collection. LSU, mSSU, and ITS sequences were obtained and a combined maximum likelihood tree of SSU and LSU sequences was constructed.

Key words—*Entolomataceae*, taxonomy, *Rhodogaster*, sequestrate fungi, genetic distance

Introduction

In *Entoloma* sporocarp morphology is highly variable: ranging in size from tiny to large and in form as pleurotoid, omphalinoid, collybioid, mycenoid, tricholomatoid, and sequestrate. In the past, three smaller non-agaricoid genera have been distinguished based on habit: *Rhodocybella* T.J. Baroni & R.H. Petersen (cyphelloid), *Rhodogaster* E. Horak (secotioid), and *Richoniella* Costantin & L.M. Dufour (gasteroid).

The first record of a secotioid entolomatoid species was published by Horak (1963) from Chile, introducing a new monotypic genus for the species *Rhodogaster chilensis* E. Horak. In 1995, a similar collection found in Spain was initially misidentified as *R. chilensis* (Calonge & Pasabán 1995) but later recognized as a new species, *Rhodogaster calongei* (Horak & Moreno 1998).

Molecular phylogenetic studies of Co-David & al. (2009) placed the sequestrate genera *Richoniella* and *Rhodogaster* within *Entoloma;* and they transferred the two *Rhodogaster* species to *Entoloma*.

Entoloma calongei was recorded during field research in Ľubovnianska Upland in NE Slovakia in 2017, and its identification has been confirmed by DNA sequence analyses. The Slovak collection represents only the second published record of this species.

Material & methods

SPECIMENS: Morphological and anatomical studies were conducted on fresh and dried basidiomata. Colours were determined according to Kornerup & Wanscher (1974). Microscopic features were studied in water or 3% KOH. All light micrographs of microscopic structures were made with a Nikon Eclipse Ni light microscope using bright field or differential interference contrast without staining, and images were captured with a Nikon DS-Ri2 digital camera. Images were processed and descriptive statistical analysis of basidiospore dimensions was produced using NIS-Elements BR 4.40 software. The voucher specimen was deposited in the herbarium of the Slovak National Museum-Natural History Museum, Bratislava, Slovakia (BRA).

DNA EXTRACTION, AMPLIFICATION AND SEQUENCING: DNA from the Slovakian *E. calongei* sporocarp was obtained from fresh material preserved in acetyl trimethylammonium bromide (CTAB) buffer and ground by using a plastic pestle and isolated by JENA Bioscience Blood-Animal-Plant DNA Preparation Kit following manufacture protocol. Target regions of the partial mitochondrial small subunit (mtSSU) rDNA and the nuclear large ribosomal subunit (LSU) were amplified using primers MS1/MS2 (White & al. 1990) and NL1/NL4 (Vilgalys & Hester 1990). The reaction was performed in 25µl of reaction mixture containing 10 pmol of both primers, Crystal Hot Start Master, and 20 ng of template DNA. The cycling programme for mtSSU consisted of an initial denaturation at 94°C for 3 min, followed by 40 cycles of 94°C 30 s, 55°C for 30 s, 72°C for 1 min, and a final extension at 72°C for 5 min and for LSU of an initial denaturation at 95°C for 3 min, followed by 32 cycles of 95°C 30 s, 55°C for 30 s, 72°C for 1 min + increasing time 2 sec per cycle, and a final extension at 72°C for 10 min. The PCR products were purified using Qiagen columns (QIAquick PCR Purification Kit, Qiagen) according to the manufacturer's recommendations and sequenced commercially by Eurofins Genomics GmbH (Cologne, Germany).

ALIGNMENT AND PHYLOGENETIC ANALYSIS: DNA sequences of *Entolomataceae* and a selected *Clitocybe* outgroup were downloaded from NCBi on 18 January 2019. These included LSU and mtSSU sequences associated with Baroni & Matheny (2011). In total 15 LSU, and 15 mtSSU sequences were retrieved, including *E. calongei* type (TABLE 1). To these, we added newly generated LSU and mtSSU sequences from *Entoloma calongei* (BRACR30482). The tree was reconstructed by the maximum-

TABLE 1. *Entoloma* and *Clitocybe* sequences used for phylogenetic analysis (newly obtained sequences in **bold**).

SPECIES	COUNTRY	LSU	SSU	ITS
C. dealbata	Unknown	AF223175	DQ825431	—
E. albidoquadratum [holotype]	India	GQ289151	GQ289291	—
E. calongei [holotype]	Spain	GQ289158	GQ289298	—
***E. calongei* BRACR 30482**	**Slovakia**	**MK531556**	**MK530244**	**MK907407**
E. cocles	Finland	GQ289159	GQ289299	—
E. costatum	Netherlands	GQ289161	GQ289301	—
E. excentricum	Germany	GQ289163	GQ289303	—
E. gasteromycetoides	New Zealand	GQ289164	GQ289304	—
E. phaeomarginatum	Tasmania	GQ289179	GQ289319	—
E. porphyrescens	Tasmania	GQ289182	GQ289322	—
E. procerum	Tasmania	GQ289183	GQ289323	—
E. serricellum	Belgium	GQ289190	GQ289330	—
E. serrulatum	Tasmania	GQ289192	GQ289332	—
E. tectonicola [holotype]	India	GQ289196	GQ289336	—
E. transmutans	Tasmania	GQ289200	GQ289340	—

likelihood method in MEGA-X with 1000 bootstrap replicates. The General Time Reversible model (Nei & Kumar 2000) with gamma-distributed evolutionary rates (G) and invariable sites (I) was chosen by MEGA as the best-fitting model. A matrix of inter-species distances was constructed using Kimura-2 parameter distances (Kimura 1980) in MEGA-X. We also produced an ITS barcode for *Entoloma calongei* (BRACR30482), now stored as SKDNA023-19 in BOLD v4 database (Barcode of Life Datasystems http://v4.boldsystems.org/).

Taxonomy

Entoloma calongei (E. Horak & G. Moreno) Noordel. & Co-David, Persoonia 23: 166 (2009) FIGS 1–3

≡ *Rhodogaster calongei* E. Horak & G. Moreno, Sydowia 50: 188 (1998)

PILEUS ≤25 mm diam., globose to subglobose (pyriform), margin involute and mostly attached to stipe, not expanding, greyish brown (8B1) with dark spots, silky and fibrillose, dry. STIPE short, with columella, ≤20 mm long, hollow, attenuated at base, silver grey (8B1), smooth, silky, fibrillose, dry. GLEBA irregularly labyrinthiform of reduced lamellae, non-gelatinized, lamellae greyish-white in section and pale pink (8B3) on surface. SMELL AND TASTE not distinctive. SPORE PRINT pink. BASIDIOSPORES cuboid or rhomboid ("pseudocuboid" sensu Karstedt & al. 2019), rarely pentagonal, (5.6–)6.3(–7.1) × (5.4–)6.8(–9.0) µm (Q = 0.80–1.01), often distorted, smooth, thin walled with distinct apiculus. Basidia 30 × 10 µm cylindrical to subclavate, 4 spored, clampless. Tramal hyphae short, cylindrical, clampless,

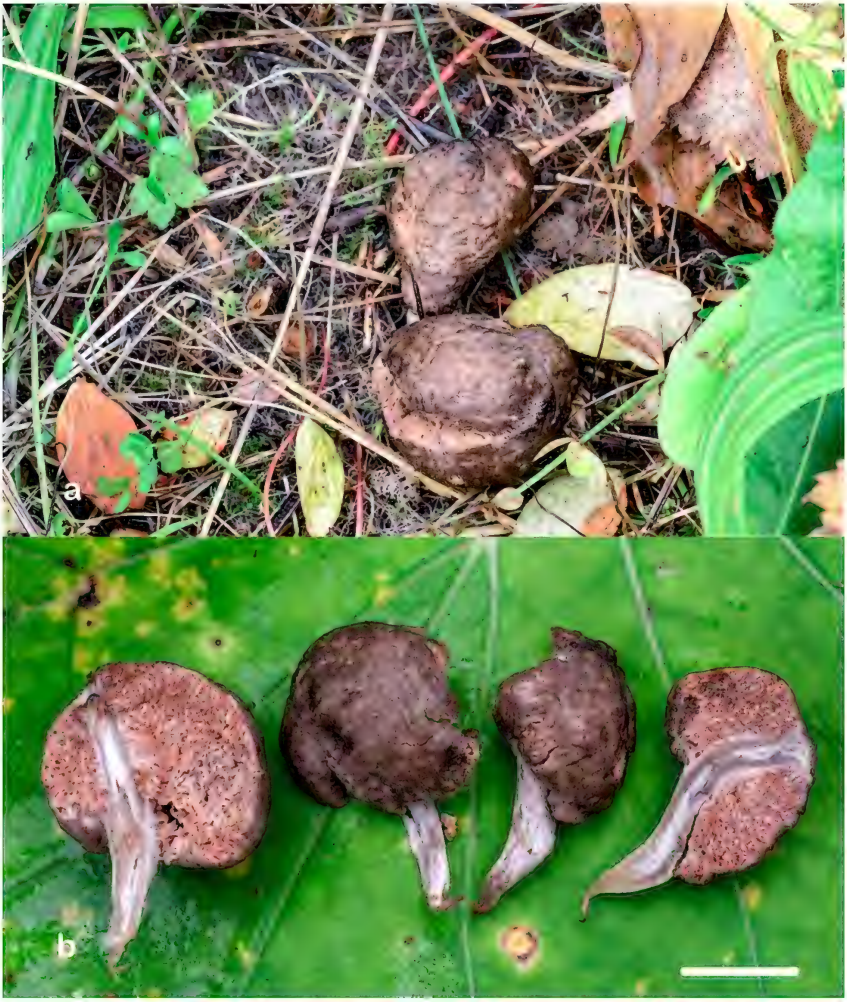

Fig. 1. *Entoloma calongei* (BRACR30482): a. In situ, 20.X.2017. (Photo: F. Fuljer); b. Sporocarps in cross section (Photo: A. Polhorský). Scale bar = 10 mm.

10–30 × 2–10 µm. Pileipellis a cutis of cylindrical, narrow (80–100 × 5–6 µm), parallel clampless hyphae, subcutis elements wider and shorter (40–80 × 15–20 µm).

Specimen examined: **SLOVAKIA**, **Prešov Region**, **Ľubovnianska Upland**, Obručné village, 49.3080°N 20.9877°E, alt. 650 m, in grass in pasture, close to mixed forest, 20. X. 2017, leg. V. Kučera, det. V. Kautman (BRACR30482).

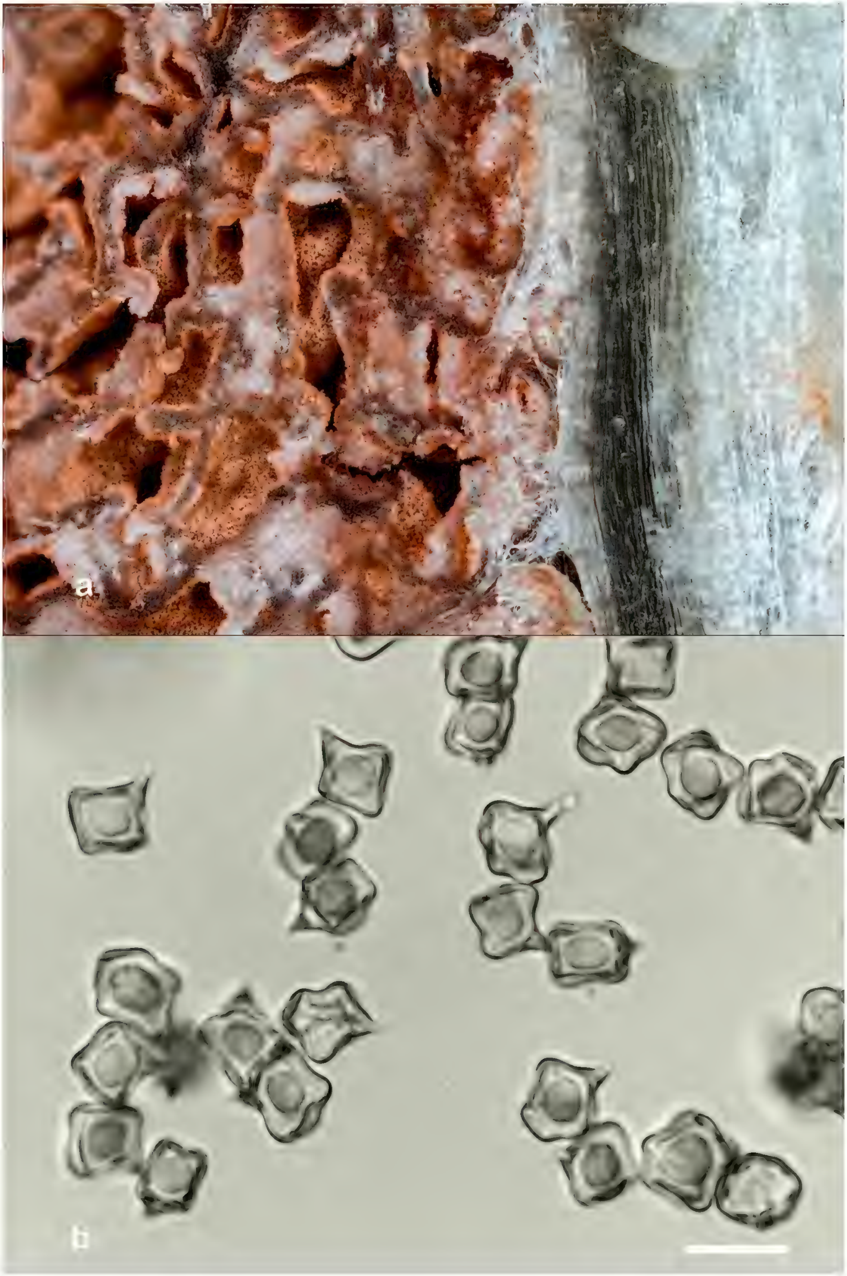

FIG. 2. *Entoloma calongei* (BRACR30482): a. Detail of gleba in cross section. (Photo: A. Polhorský); b. Spores (Photo: I. Kautmanová). Scale bar: a = 1 mm; b = 10 µm.

HABITAT—Seminatural meadow mowed and/or grazed, southern slope; soil flysch sandy-silty, slightly basic but not calcareous. The specimens grew in grass, herbs (*Plantago*, *Trifolium*, *Taraxacum*), and mosses, ca 50 m from the edge of mixed forest (*Picea abies, Carpinus betulus, Fagus sylvatica, Corylus avellana, Cornus sanguinea, Betula, Acer*). The species was found in a rich grassland-fungi community together with various earth-tongues, waxcaps, and clavarioid fungi.

Discussion

Vidal & al. (2016) reported a total of 11 sequestrate *Entoloma* species, including four from Europe/Spain. Nine species were gasteroid and only two secotioid: *E. calongei* and *E. chilense* (E. Horak) Noordel. & Co-David. These two species differ from each other in a number of basic characters, including basidiospore morphology and pileipellis structure.

Basidiospores of *E. chilense* were described as "ellipsoidnodular" (9.5–11.5 × 7.5 µm; Horak 1963), and those of *E. calongei* as cuboid (side lengths 6–10 µm; Horak & Moreno 1998). Basidiospores of our collection were cuboid or rhomboid, often broader than long and side length is 6–8 µm.

Although Horak (1963) described clamp connections as absent on basidia and tramal and cortical hyphae in the type description of *E. chilense*, Horak & Moreno (1998) later reported regularly observing clamp connections in *E. chilense,* contrasting with the clampless *E. calongei.* No clamp connections were observed in our specimens.

The pileipellis in the Slovak collections is a cutis composed of cylindrical parallel hyphae, the same as cited for *E. calongei* in the protologue and differing from the trichoderm of *E. chilense* (Horak 1963).

Ecology of the type localities of the two taxa was distinctly different. *Entoloma chilense* occurs among deep litter and humus in the coastal anectotrophic rain forest of Central Chile; the vegetation around the type locality was dominated by trees in *Lauraceae* and *Myrtaceae*, with *Chusquea* sp. (*Bambuseae*) in the understory (Horak 1963). By comparison, *E. calongei* was found in northern Spain in a colline-temperate mixed forest of conifers (*Larix*, *Pinus*) and broadleaf trees (*Fagus*, *Acer*, *Quercus*, *Fraxinus*, *Corylus*) at about 700 m a.s.l. (Calonge & Pasabán 1995). Our Slovakian collection site differed in that the basidiomata grew among grass and mosses in a pasture; however, the site was only 50 m from a forest composed of conifers and broadleaf trees similar to those in Spain.

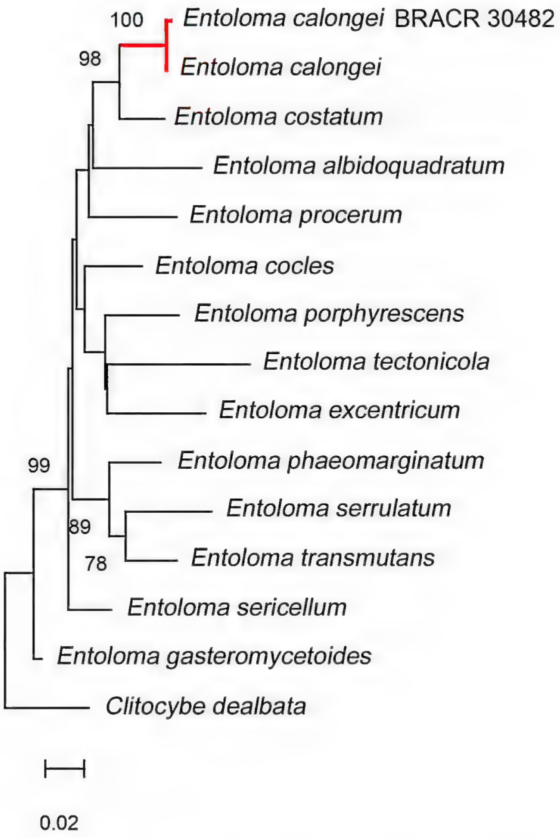

Fig. 3. Maximum likelihood phylogenetic tree constructed from the combined dataset (LSU+mtSSU). Red clade represents well-delimited species *Entoloma calongei*. *Clitocybe dealbata* was used as outgroup. Numbers above branches represent bootstrap support (only values >50% are shown).

It should be added that many *Entoloma* species occur both in semi-open forests and in grasslands without trees (Noordeloos 1992, 2004).

The sequence of *E. calongei* from Slovakia is almost identical with the type and the identity of Slovak collection has also been confirmed by using Kimura-2 parameter distances model. Our phylogeny (Fig. 3), like that of Co-David & al. 2009, places *E. calongei* close to *E. costatum* (Fr.) P. Kumm. with c. 3% dissimilarity (Table 2). *Entoloma costatum* is an extremely rare, rather fleshy, clitocybeoid species with rhomboid-

TABLE 2. Matrix of genetic inter-species distances from the *Entoloma* and *Clitocybe* sequences.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	*E. calongei* Slovakia	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
2	*E. calongei* Spain [Type]	0.001	—	—	—	—	—	—	—	—	—	—	—	—	—	—
3	*E. costatum*	0.043	0.035	—	—	—	—	—	—	—	—	—	—	—	—	—
4	*E. albidoquadratum*	0.062	0.060	0.059	—	—	—	—	—	—	—	—	—	—	—	—
5	*E. procerum*	0.065	0.055	0.052	0.065	—	—	—	—	—	—	—	—	—	—	—
6	*E. cocles*	0.054	0.047	0.052	0.066	0.051	—	—	—	—	—	—	—	—	—	—
7	*E. porphyrescens*	0.057	0.053	0.055	0.063	0.063	0.049	—	—	—	—	—	—	—	—	—
8	*E. tectonicola*	0.079	0.078	0.082	0.092	0.076	0.075	0.064	—	—	—	—	—	—	—	—
9	*E. excentricum*	0.069	0.065	0.063	0.063	0.061	0.059	0.057	0.074	—	—	—	—	—	—	—
10	*E. phaeomarginatum*	0.066	0.055	0.054	0.060	0.050	0.049	0.056	0.076	0.065	—	—	—	—	—	—
11	*E. serrulatum*	0.081	0.070	0.075	0.080	0.067	0.063	0.067	0.083	0.072	0.051	—	—	—	—	—
12	*E. transmutans*	0.060	0.053	0.051	0.061	0.054	0.046	0.051	0.067	0.061	0.040	0.044	—	—	—	—
13	*E. sericellum*	0.047	0.042	0.035	0.053	0.044	0.038	0.044	0.069	0.052	0.041	0.060	0.041	—	—	—
14	*E. gasteromycetoides*	0.049	0.043	0.042	0.061	0.048	0.039	0.039	0.069	0.057	0.042	0.060	0.041	0.030	—	—
15	*C. dealbata*	0.078	0.068	0.074	0.081	0.077	0.071	0.071	0.103	0.081	0.065	0.087	0.066	0.055	0.045	—

cuboid spores (Noordeloos 2012). However, according to Karstedt & al. (2019), *E. calongei* is closer to *Entoloma asterosporum* (Coker & Crouch) T.J. Baroni & Matheny [≡ *Richoniella asterospora*]. These three species form one of many independent clades of *Entoloma* species possessing cuboid spores (Karstedt & al. 2019). However, the three differ from the others in having a special "pseudocuboid" spore-shape (Karstedt & al. 2019). Co-David & al. (2009) and Karstedt & al. (2019) place these species basal to the Inocephalus–Cyanula superclade, with weak support.

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